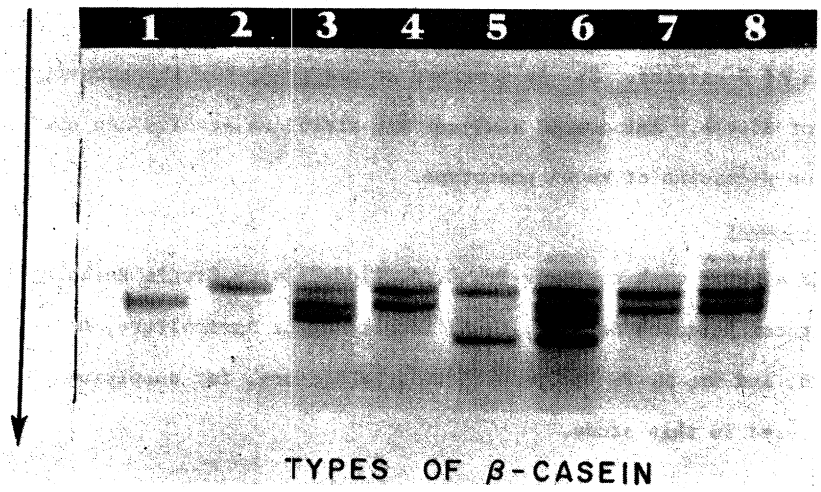
the buffer vessels are filled with 1.33 M (7.7%) acetic acid. The negative electrode is connected to the lower buffer vessel. The cell is then run without sample, but with cooling at a current of 95 ma for 2 hours. The cooling was standardized at 14° C.

The samples are dissolved in the stock buffer, to which urea has been added to make it 10 M in urea. Methyl red is used to assist in observing the layering of the sample. The β-casein is dissolved to 0.25% concentration in this solution; for a gel with eight slots, 0.015 ml per slot is added. For typing of whole casein, 0.015 ml of 1% solution is added per slot.

After the casein solutions have been added, the cell is run with only 25 ma current for 20 minutes, then the current is raised to 100 ma. The voltage is about 150 v with the constant voltage power supplies used. As the current decreases, the voltage is increased to keep the current at 100 ma until 300 v is reached. Then the power supply is left at this setting, and the current gradually drops to about 60 ma at the end of the run. At the end of 20 hours the casein samples are sufficiently resolved for typing.

The gels are removed and dyed in a saturated solution of Naphthol Blue Black (C. I. 20470) in a mixture of 50 parts methanol, 50 parts water and 10 parts acetic acid. After staining for 20 minutes the excess dye is removed by a succession of baths of the same solvent or electrolytic destaining. Fig. 1 is a photograph of such a run.

We suggest that the β-caseins be renamed A, B, C, D, and E from their order in acid gel electrophoresis where A has the greatest mobility and the others follow in decreasing order of mobility. β-Casein, typed C in alkaline electrophoresis becomes A and β-casein B remains B in the proposed nomenclature. C, D, and E are those β-caseins which were indistinguishable by alkaline electrophoresis and were typed as A. The β-casein A of Table I is now C and the β-casein A' is now type D. The β-caseins shown were separated by the urea fractionation method only. Column chromatography removes the minor components. In the eight slots of the gel (Fig. 1) the resolution of the method is demonstrated. In slot



6 an artificial mixture of the four types A, B, C, and D was placed. All of these have been found to exist in individual milks as single types. Type E is is not represented in the photograph, since it was discovered only recently, and only caseins containing mixtures of E and D or E and C are available now. In slot 1 a β-casein of type C is demonstrated; slot 2 is a β-casein with only type D. Slot 3 contained an artificial mixture of the B, C, and D types. Slot 4 was a mixture of types C and D, and slot 5 was a single β-casein which contains both A and D types. Slot 7 contained a β-casein obtained from pooled milk which was typed as A in alkaline gels and had been used in structure work. Slot 8 contained a single β-casein which has both types C and D. A paper on the mode of inheritance of the new types of β-casein is in preparation. Of 63 caseins typed in acid gels to date, the phenotypes observed were as follows: BC, 3; BD, 3; C, 3; CD, 33; CE, 3; D, 17; and DE, 1. No resolution of the genetic variants of α_{s_1}-caseins or κ-caseins has been noted in the small number of samples typed in acid gels to date.

It can be concluded that A type β-casein typed by the alkaline method represents a mixture of six phenotypes, and that structural work should not be undertaken on β-caseins derived from a mixture of such milks.

This discovery of additional polymorphism in the β-caseins is valuable

for the following reasons: 1. As an explanation for divergence of amino acid analyses of β-caseins. 2. As a method of searching for the ancestry of various breeds of cattle. Amino acid analyses and structure studies can now be performed on β-caseins of known phenotype.

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DETECTION OF NEW TYPES OF β-CASEIN BY POLYACRYLAMIDE GEL ELECTROPHORESIS AT ACID pH: A PROPOSED NOMENCLATURE

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Current nomenclature (Thompson et al., 1965) of the genetic polymorphs of β-caseins (A, B, and C) was established from information obtained by examining individual cow caseins by paper electrophoresis or starch gel and polyacrylamide gel electrophoresis at alkaline pH's (Aschaffenburg, 1961; Peterson, 1963; Thompson et al., 1964; Aschaffenburg, 1964). Chemical work in this laboratory, however, had shown inhomogeneity existed in β-casein separated from pooled milk, with respect to histidine variation in a molecular weight 6,700 peptide isolated from a trypsin hydrolysate (Nauman et al., 1960). To find if the histidine variation was present in the A or B or C type, β-caseins A and B were prepared from the milk of cows which were homozygous with respect to A and B β-casein, and C β-casein was separated from an A/C β-casein by column chromatography (Ribadeau-Dumas, 1961). The β-caseins were first separated by the urea fractionation method (Hipp et al., 1952), then repeatedly chromatographed until they gave only one band in acrylamide gel electrophoresis. The histidine variation was finally associated with A type β-caseins (Peterson and Nauman, 1963) which were chemically different, but could not be differentiated by gel electrophoresis at alkaline pH's.

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A complete analysis of the β-caseins analyzed in the course of the work will be published elsewhere, however, the analytical difference in the basic amino acids can be used to distinguish the two A types and the B and C β-caseins, as summarized in Table I:

TABLE I

Basic amino acid content of the genetic variants of β-casein
Amino acid residues per 24,100 MW

Phenotype	Lysine	Arginine	Histidine
A	11	4	6
A'	11	4	5
B	11	5	6
C	12	4	6

If electrophoresis is conducted in formic acid-acetic acid buffer at pH 3.0 the four β-caseins in the table can be resolved from mixtures. In addition, still another β-casein, also typed as A β-casein by alkaline gel electrophoresis, can be distinguished. The typing method is reported at this time to advise other workers in the field that "A" type β-casein may be inhomogeneous where the typing has been by the alkaline buffer methods.

The vertical water-cooled cell of Raymond (1962) is used for the typing. The slot formers should be shortened or coated with a film of silicone grease to aid in their removal from the 10% gels. The stock buffer is made by diluting 86 ml of glacial acetic acid and 25 ml of 90% formic acid to 1 liter. For each gel, 15 g of Cyanogum-41 and 40.5 g of urea are dissolved in the buffer to make 150 ml of solution. This is warmed to 25° C, 1.0 ml of TEMED (N,N,N'N'-tetramethylethylenediamine) is added followed by addition of 0.35 g of ammonium persulfate. The gel mold is not cooled at this point. Polymerization is apparent at 20 minutes, but it is best to wait for an hour before removing the slot formers. The cell is then turned so the gel is vertical and